EUROPEAN COOPERATION PROGRAM INTERREG VA GREECE - ITALY 2014/2020 - BEST PROJECT. PROCEDURE EX ART. 1 OF DLN 76 OF 16/07/2020 CONVERTED INTO LAW N. 120 OF 11/09/2020 AND PURSUANT TO ART. 95, PARAGRAPH 3 OF D.LGS. 50/2016 FOR THE ASSIGNMENT OF THE SERVICE OF "ANALYSIS OF AGROBIODIVERSITY AND STUDY OF VEGETABLE SPECIES GROWN AT RISK OF EXTINCTION IN THE AREA OF PILOT ACTION 1 OF THE BEST PROJECT AND RELATED ACTION PLAN". CUP: B38H19005670006 - CIG: 8730686601.

Report on the activities of the contractor GAL SEB scarl referred to point c) of art. 4 - TERMS FOR CARRYING OUT THE ACTIVITIES of the contract:

- **descriptive document relating to the morpho-physiological characterization of the plant species subject to analysis, art. 1 point 2 lett . c);**
- **preliminary version of the database containing the acquired data, the results of the characterizations, the information about the in situ / ex situ conservation of cultivated plant species, art. 1 point 2 lett . f);**

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Summary

1.0 M ATERIALS AND METHODS

The characterization of the plant species was performed on typical specimens of the 3 BEST project areas, belonging to the list drawn up during the two phases that preceded this report. The analysis was conducted on the basis of objective and shared criteria in a scientific reference framework and according to common and harmonized procedures at national and international level such as those proposed in the guidelines for the conservation and characterization of plant, animal and microbial biodiversity. interest in agriculture. The guidelines set out in the National Plan of Agricultural Interest (PNBA) were created by the Ministry of Agricultural, Food and Forestry Policies as part of the program of activities for the implementation of the National Plan for Biodiversity of agricultural interest (Ministerial Decree 28672 of 14 / 12/2009), under the supervision of the Standing Committee for Genetic Resources in Agriculture. This summary document shows the methods of data collection, the main descriptors and the methods of processing through appropriate statistical analyzes for the correct characterization of Plant Genetic Resources (RGV) of interest for agriculture.

The characterization is aimed at the precise identification of a RGV. The Working Group Biodiversity in Agriculture (GlBA) presented the most effective descriptors divided into categories, illustrating the guidelines for their use. The work starts from the evaluation of single accessions to arrive, where possible, at the constitution of a variety card that summarizes the morpho-physiological profile of the variety starting from the observation of single accessions. Sometimes the local varieties, especially if herbaceous, are characterized by a certain internal diversity, which evolving in space and time (both for environmental and anthropic action), also makes them unstable. When these characteristics are particularly accentuated, it is not possible to fully use the characterization tools developed on the improved varieties (typically uniform and stable). In these cases it is necessary to resort to the evaluation by single plant, to identify subpopulations or varietal typologies through the attribution of frequency classes. On the other hand, when the local variety shows a low level of internal variability, it is possible to apply the characterization systems developed to evaluate the DUS (Distinguishing , Uniformity and Stability) criteria. These criteria, albeit with greater flexibility, are also indispensable for the purposes of registration in the National Register of conservation varieties. Collection of information on existing local varieties. A first description of the RGVs found in the area is the initial phase of a conservation process. This is followed by a more precise in situ / on farm or ex situ characterization depending on the conservation model. The GlBA has defined some forms and elaborated models, able to cover all the information gathering and characterization needs of the RGV. Overall, the method proposed in the PNBA allows to implement the phases of characterization, organization, coordination and monitoring of the conservation activities described. It should be considered that even single parts of the general scheme can be created, thus adopting a certain flexibility in the use of the tools proposed in the plan.

The passport descriptors (i.e. those identifying a RGV referring to the precise conditions of retrieval) are essential to unequivocally identify and distinguish each accession, even when it is propagated or transferred. These passport descriptors are also those which, as provided with common coding systems from international databases (MCPD and EURISCO), allow comparison with materials held in other countries. In addition to the internationally coded passport descriptors, the GLBA , having heard the opinion of the regional delegates, proposed four further additional and complementary identification descriptors,

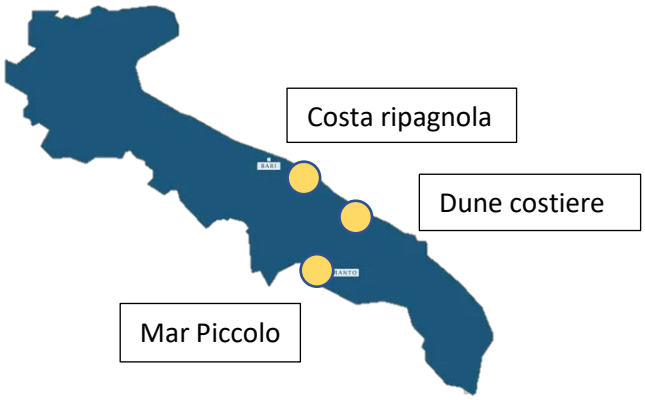
considering that they could provide useful information of local or national interest for a more detailed identification of accessions. Finally, two other particular descriptors have been reported, which identify those accessions that are designated as belonging to the species of Annex I of the International Treaty and / or as components of the European Collection defined within the European Integrated System of Genetic Banks.

1.1 Morpho-physiological characterization

The description of the phenotype of plants represents one of the most important tools for investigating biodiversity. This description, based on the survey of morpho-physiological characters, allows to characterize, distinguish and identify the varieties, using appropriate comparison methods. Descriptors generally refer to highly heritable and stable traits and often also constitute the basic elements of the taxonomic classification of plants. The characterization must be carried out with objective and shared criteria, in a scientific reference framework and possibly according to common and harmonized procedures at national and international level. The GlBA has proposed a descriptive file (defined as species-specific) for the description of a local variety or accessions of a local variety within the considered species. If the characterization is aimed at identifying the variety, generally all the characters provided for in the descriptive sheets must be used and systematically detected according to the indicated procedures. At an international level, various systems have been developed aimed at varietal characterization and specifically dedicated to the description, documentation, exchange and management of genetic resources (Bioversity International, USDA-GRIN) or to the assessment of the requirements of distinguishability , homogeneity, stability and uniqueness. required for the issue of varietal protection titles (CPVO, Community Plant Variety Office). In relation to the objectives set for most of the species, the international system of UPOV (Union Internationale pour la Protection des Obtentions Végétale) and therefore it is generally referred to in the illustrated varietal characterization methodologies. The basic criteria of the UPOV international system are consistent with the national and European system of official varietal registration, are known and already in use for many species by different Regions and are considered substantially corresponding with the international IPGRI / Bioversity system of descriptors of characterization. In the case of some species, including the vine, other organisms - such as the Organization Internationale de la Vigne et du Vin (OIV) - worked together with UPOV and Bioversity in the creation of a system of common descriptors of the genus Vitis . Since it is the most used system for vines at regional, national and international level, the form for the morpho-physiological characterization of the Vitis vinifera species refers to these descriptors. Finally, other descriptors have been elaborated and introduced in the proposed files on the basis of the experiences of the members of the GlBA . In the species propagated by seed it is also important to keep in mind - as mentioned in the introduction - that the local varieties do not have the same characteristics as the improved varieties, on which the UPOV and CPVO criteria have been calibrated. In fact, they are often characterized by high internal variability and therefore some procedures envisaged by these Bodies (for example those relating to the evaluation of "homogeneity") are not always applicable.

Descriptive morphological sheet

The form used for the identification and characterization of the varieties taken into consideration in the BEST project is proposed below.

RGV	Type of genetic resource
Genetic resource	**Common name of the characterized variety**
Conservation bodies	Entities that own and / or conserve the plant or parts of it for its multiplication (mother plants, seeds)
In vivo conservation	Type of in vivo conservation applicable
In vitro conservation / germplasm banks	Applicable in vitro storage type
Possible start-up in BEST areas	
(photo)	
Plant	Type of growth of the plant, height, luxuriance
Leaves	Color, shape, size and texture of the leaves
Flowers	Number of flowers, size, color, etc.
Fruits	Characteristics of the fruit and seeds.
Production characteristics	Productivity and agronomic aspects
Other technological features	Organoleptic, chemical and nutraceutical aspects

1.2 Molecular characterization.

From their first applications in the plant field a little over twenty years ago, molecular markers have proved to be increasingly promising and useful tools for investigating genetic diversity, thanks to the growing progress in knowledge of the genome of organisms and the consequent development of increasingly

effective and less expensive analytical techniques. Indeed, each individual has differences in their DNA which, albeit minor, distinguish them from other individuals of the same species and / or population. Such polymorphisms can be detected by comparing homologous stretches of DNA between individuals. This is the analysis of the so-called molecular markers, or DNA fragments positioned in points of the chromosome (therefore inheritable), which with their presence uniquely distinguish ("mark") the stretch of DNA in which they are found. It is evident that the characterization of the genotype through the analysis with molecular markers presents, compared to the morphological description of the phenotype, undoubted advantages, including that of avoiding the interference of the environment in the expression of characters and the inevitable subjectivity of morphological findings, thus offering greater reliability in the event of legal disputes. Furthermore, DNA analysis can detect differences even between genetically very similar individuals (often not phenotypically distinguishable) and, due to the inheritance of the markers, offer objective information on the genetic proximity between individuals or populations and on the identification of parents (pedigree) whenever it is important to establish / confirm the genetic origin of a variety. DNA can be extracted from many parts of the plant (stem, leaves, fruits, seeds, roots), during the vegetative cycle or during the winter rest, and has the advantage of being a relatively stable and storable molecule. The aforementioned positive aspects, combined with the development of analytical techniques and instrumentation with increasingly sustainable costs, make molecular markers increasingly widespread tools, however capable not so much of replacing, but of profitably supporting the morpho-physiological descriptions in the characterization of RGV, noting differences at the DNA level where morpho-physiological markers fail. A good knowledge of the phenotypic variability of the species is always essential both in the sampling of the material and in the interpretation of the results obtained with genetic analyzes. Furthermore, while molecular markers of great efficacy in the distinction between individuals, in varietal identification and in the study of genetic relationships have been studied for some crops (and databases of reference genetic profiles are also beginning to be available for operators), for others species, on which the attention of the scientific community has not been concentrated, the methods available are scarce, not particularly informative or even nil. Among the crops of the first type we must undoubtedly remember the grapevine, for which some widely used microsatellite markers have been adopted as genetic descriptors and, after setting up a system for coding the results to standardize the data coming from laboratories several, added to the official list of morpho-physiological descriptors of international use for the characterization of vine species and varieties. Databases of genetic profiles of European grape varieties are now accessible online and are periodically updated. In summary, it can be said that practical and field skills on the morphology and physiology of the species to be characterized are irreplaceable, while genetic methods can usefully come into play in the objective confirmation of varietal identities on the basis of a precise genetic profile of reference, very much indicated for example in the case of errors in the denomination of varieties or synonyms between cultivars present in distant places. Finally, molecular markers can provide scientific information of great importance in the management and study of RGV, such as in the constitution of the so-called core collections (collections that contain the widest genetic diversity in a limited number of individuals) or in the definition of genetic variability. of a population and its structure and, more generally, in the assessment of the risk of genetic erosion and in the monitoring of the effectiveness of conservation interventions.

For a better understanding of the scientific aspects that characterize this paper, a short list of definitions relating to the terminology used is provided.

DNA = deoxyribonucleic acid. It is the seat of the genetic information of every living being. Its code is universal, the molecule is formed by a double helix of nitrogenous bases, phosphate groups and sugars. It is a stable molecule, resistant to high temperatures (it denatures at 95 ° C), to industrial processing and is found both in raw materials (olives) and in derived products (oil).

PCR = stands for the technique called Polymerase Chain Reaction . It is the molecular biology technique that most of all has revolutionized the world of research. Thanks to the characteristics of a particular enzyme, polymerase, and to cycles of lowering and thermal raising, it is possible to open the DNA double helix, and highlight a particular DNA fragment of our interest, favoring the formation of millions of copies of the fragment in so you can study and analyze it.

MOLECULAR MARKER = the molecular marker is a sequence (set of nitrogenous bases) of DNA that serves to identify a specific region to be studied. For many plant and animal species, the complete sequence of the entire genome is not available, nor is the location and function of the genes exactly known, for these reasons, to highlight the differences in the DNA sequence of two individuals belonging to the same species, it is necessary to use molecular markers.

SSR or MICROSATELLITE MARKER = microsatellite markers or SSR (simple sequence repeats) are molecular markers that are commonly used in identification studies of individuals, in the reconstruction of family ties, in forensic genetics, in the traceability and traceability of foods. They are present in many species, including higher plants, and are distributed in the genome, mainly in non-coding sequences (part of the genome that does not contain genes but sequences that are not transcribed and translated). Their mechanism of action is as follows: each individual belonging to a specific species contains different microsatellite sequences . Microsatellite sequences are short repetitions of nitrogenous bases (the nitrogenous bases of DNA are Adenine, Thymine, Cytosine, Guanine) usually in tandem: AT-AT-AT-AT-AT. The basic motif (AT) is repeated in individuals of the same species a different number of times among different individuals. The different number of repetitions causes a length polymorphism. Length polymorphism means that the DNA fragment identified by the microsatellite marker in individual 1 is different from individual 2. The length of the fragments is expressed in bp (base pairs). These markers typically show fragments of length between 100 and 500 base pairs, therefore very small, are highly reproducible, have a high level of polymorphism, are

widely used by scientific literature, are extremely reliable, suitable for use with starting DNA fragmented. The microsatellite marker is based on the PCR technique.

ALLELE = alternative form of a DNA sequence. In the case of the microsatellite marker , the allele is represented by the length of the microsatellite sequence found in different individuals.

Laboratory activities

The laboratory activity involved the molecular characterization of different varieties of fig, plum, vine, almond and pear tree by means of microsatellite analysis . Table 1 lists the varieties under study. It was preferred to carry out the molecular analysis on genotypes belonging to arboreal plants, because there is a greater certainty of finding them stably in the analyzed territories, because the propagation takes place vegetatively, because the variety is identified with the concept of clone and therefore the material genetic belonging to a given variety is stable over time (unless it is subject to the natural action of mutations), and consequently its molecular profile (fingerprint) remains unaltered. Unlike what is reported in the text of the project announcement, the choice of the molecular marker to be used fell on the microsatellite and not on the DNA barcoding , because the latter is not able to easily detect polymorphisms within the same species but between species different. Compared to the 15 predicted genotypes, 23 have been characterized, shown here in table 1

Table 1. List of varieties analyzed using microsatellite markers (SSR) to obtain molecular profiles

Specie	Varietà
Fico (*Ficus carica L.*)	Petrelli
	Verdesca
Susino europeo (*Prunus domestica*)	Sant'Anna
Susino indogiapponese (*Prunus salicina*)	Goccia d'oro
Vite (*Vitis vinifera*)	Cigliola
	Notardomenico
	Santa Teresa
	Ignota Bianca
	Piccola nera
	Pizzutella
	Lattuario
	Giulia Ciola
	Chiobbica
	Sagrone rosso
Mandorlo (*Prunus dulcis* Mill. D.A. Webb.)	di Sabato
	Montranese
	Montrone
Pero (Pyrus communis L.)	Pero Gentile Reale
	Pero Recchia Falsa
	Pero a sole
	Pero Campanello rosso di Ottobre
	Pero San Giovanni
	Pero Genio

The protocols used for obtaining nucleic acids from plant material (leaves) for the species examined and characterized during this activity are listed below.
Specifically, these are extraction protocols of good applicability and capable of providing a high yield of good quality DNA starting from low quantities of plant material.

Extraction of Fig and Pear DNA by Doyle & Doyle modified protocol , 1991

1. Pulverize 50 mg of lyophilized leaf tissue
2. Add to each sample 700 µl of CTAB extraction buffer (2% CTAB, 1.4 M NaCl , 0.2% β - mercaptoethanol , 20 mM EDTA, 100 mM Tris-HCl, pH 8.0)
3. Incubate the sample at 60 ° C for 30 (15-60) minutes, shaking gently from time to time
4. Add 700 µl of chloroform- alcoholisoamyl (24: 1), mixing gently

5. Centrifuge at 4 ° C to separate the phases at 11,000 x rpm for 10 minutes
6. Collect the supernatant and repeat the chloroform- alcoholisoamyl wash (24: 1) and re- centrifuge at 11,000 x rpm for 10 minutes at 4 ° C
7. Take the supernatant, transfer it to an eppendorf and add 2/3 volumes of cold isopropanol and mix gently to favor the precipitation of nucleic acids
8. Incubate the samples at -20 ° C over-night
9. Centrifuge at 11,000 x rpm for 15 minutes at 4 ° C
10. Discard the supernatant and wash the pellet with 76% ethanol
11. Centrifuge 11,000 x rpm for 3 minutes at 4 ° C
12. Discard the supernatant taking care not to lose the pellet
13. Spin and remove excess ethanol
14. pellet to dry
15. Resuspend 30-50 µl of TE 0.1X

DNA Extraction of Plum, Vine and Almond Tree by Spadoni et al. Protocol, 2019

1. Approximately 100 mg of pulverized leaf tissue was resuspended in 900 µL of preheated extraction buffer, then incubated at 65 ° C for 30 minutes and stirred by inversion every 10 minutes. The samples were left to cool for 5 minutes at room temperature
2. After the addition of 1 volume of cold chloroform- alcoholisoamyl (24: 1), the samples were centrifuged at 9,400 × g for 10 minutes
3. The supernatant was collected, transferred to a new 1.5 mL eppendorf , and 1 volume of cold chloroform- alcoholisoamyl (24: 1) was added, mixed gently by inversion and centrifuged again at 11,400 × g for 10 minutes.
4. Subsequently, the supernatant was transferred to a new 1.5 mL eppendorf containing 1 volume of cold 2-propanol and 0.2 volumes of Acetatmix , mixed by inversion and incubated for 30 minutes at -80 ° C.
5. The tubes were centrifuged at 6,000 × g for 5 minutes, immediately followed by 10 minutes at 9,400 × g to improve pellet deposition .
6. The pellet was washed with 700 µL of cooled 70% ethanol and centrifuged at 4,700 × 3 for 5 minutes.
7. The pellet was first dried under vacuum for 15 minutes, then suspended in 500 µL of TE buffer and added to 1 volume of phenolCIA . The tubes were mixed by inversion and centrifuged for 10 minutes at 3,400 × g.
8. The supernatants were collected and subjected to a new precipitation step by adding 2.5 volumes of cooled absolute ethanol, followed by an incubation step at -80 ° C for 30 minutes.
9. The final steps consisted of centrifuging the sample at 11,400 × g for 10 minutes and washing the sample with 500 µL of 70% ethanol.

10. After a short centrifugation (3 minutes) at 11,600 × g, the samples were dried under vacuum for 15 minutes and subsequently eluted in 50-300 µL of TE buffer.
11. To remove the RNA, 1 µL of RNase A 100 ng / µL was added to the DNA solution and the samples were incubated at 37 ° C for 30 minutes.

Evaluation of the extracted DNA

The quality and quantity of the extracted DNA were evaluated with the spectrophotometric reading at the Nanodrop , from which three values are obtained: the DNA concentration, the protein content and the polyphenol content. The last two figures are calculated based on two ratios:

- the ratio between the absorbance values at 260 nm and 280 nm indicates any contamination by proteins (for DNA this ratio must be higher than 1.7-1.9);

- the ratio between the absorbance values at 260 nm and 230 nm indicates any contamination by polyphenols and polysaccharides (for DNA the ratio must be greater than 1.8)

All DNA concentrations obtained were normalized to 70 ng / µL .

A further quantitative-qualitative control was performed on the extracted DNA, to observe any degradation and pollution due to the salts used during the extraction. This evaluation was estimated by comparing 12 µl of 1: 5 diluted DNA with a λ-DNA marker, at a known concentration on 1% agarose gel in 1X TBE buffer (0.04 M Tris-acetate, 0.001M EDTA) at 100 V for 1 hour.

SSR analysis by PCR amplification

PCR amplification was performed using specific SSR markers representative of the genome of each species examined. Primers marked Fam (blue), Vic (green) Ned (yellow) Pet (red) were used to optimize the analysis times .

Table 2 shows the SSR markers used for the molecular characterization of the varieties under analysis.

Table 2 . SSR markers used for molecular characterization

Varietà	Marcatori SSR
Fico Petrelli	MFC1, MFC3, MFC25, MFC26, MFC27, MFC28, MFC30, MFC31, MFC36, MFC37, MFC38, Cup27-4, Cup38-6
Fico Verdesca	
Susino Sant'Anna	CPDCT025, CPSCT018, UDP98-409, CPPCT006, UDP96-005, BPPCT001, BPPCT025, UDP98-412, CPSCT012, BPPCT-014, Pchgms1, ps08e08, BPPCT-010
Susino Goccia d'oro	CPDCT025, CPSCT018, CPPCT006, BPPCT001, BPPCT025, BPPCT007, UDP98412, CPSCT012, BPPCT014, Pchgms1, ps08e08, BPPCT010
Vite Cigliola	VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VRZAG62, VRZAG79
Vite Notardomenico	
Vite Santa Teresa	
Vite Ignota Bianca	
Vite Piccola nera	
Vite Pizzutella	
Vite Lattuario	
Vite Giulia Ciola	
Vite Chiobbica	
Vite Sagrone rosso	
Mandorlo di Sabato	UDP98-409, CPPCT033, CPDCT025, CPPCT006, CPDCT045, CPSCT018, BPPCT007, UDP98-412, UDP96-005, CPSCT012, BPPCT001,
Mandorlo Montranese	BPPCT025, pchgms1, UDP96003, BPPCT-010, BPPCT-014, EPPCU5176, CPDCT042
Mandorlo Montrone	
Pero Gentile Reale	CH05c06, EMPc117, GD147, EMPc11, CH03d12, CH01d09, GD96, CH02b10, CH04e03, CH01f07a, CH03g07, CH01d08, GD142, CH_Vf1
Pero Recchia Falsa	
Pero a sole	
Pero Campanello rosso di Ottobre	
Pero San Giovanni	
Pero Genio	

Below, for each species characterized by SSR markers, the composition of the PCR reaction mixture used and the amplification conditions are reported:

1. ***FIG***

   The reaction mixture used, in a final volume of 20 µl , was composed of: 150 ng / µl of DNA, 10X buffer; dNTP 200 µM , primer forward labeled with fluorophore and prime reverse 1 µM , Taq DNA Polymerase 0.05 U / µl .

   The amplifications were carried out on a C1000 Biorad thermal cycler with a touchdown protocol under the following conditions: 5 minutes at 95 ° C; 20 cycles from: 30 sec at 95 ° C, 45 sec at 60 ° C (annealing temperature), 40 sec at 72 ° C; 25 cycles from: 30 sec at 95 ° C, 30 sec at 50 ° C (annealing temperature), 40 sec at 72 ° C; final phase: 15 min at 72 ° C; stand-by at 10 ° C.

2. ***Plum***

   The reaction mixture used, in a final volume of 12.5 µl , was composed of: 50 ng / µl of DNA, 1X buffer, dNTP 200 µM ; primer forward labeled with fluorophore and reverse primer 0.25 µM , Taq DNA Polymerase 0.05 U / µl .

   The amplifications were carried out on a C1000 Biorad thermal cycler with a standard protocol under the following conditions: 5 minutes at 95 ° C; 35 cycles from: 45 sec at 95 ° C, 45 sec at 56 ° C (annealing temperature), 45 sec at 72 ° C; final phase: 8 min at 72 ° C; stand-by at 10 ° C.

3. ***Vine*** (Fanelli et al., 2021)

   The reaction mixture used, in a final volume of 25 µl , was composed of: 50 ng / µl of DNA, buffer 1X, dNTP 0.1 mM , forward and reverse primer mix 0.25 uM (with forward labeled with fluorophore), DNA Polymerase 0.05 U / µl .

   The amplifications were carried out on a C1000 Biorad thermal cycler with a touchdown protocol under the following conditions: 5 minutes at 94 ° C; 10 cycles from: 45 sec at 94 ° C, 45 sec at 60 ° C (annealing temperature), 30 sec at 72 ° C; 25 cycles from: 45 sec at 95 ° C, 45 sec at 55 ° C (annealing temperature), 30 sec at 72 ° C; final phase: 15 min at 72 ° C; stand-by at 10 ° C.

4. ***Almond tree*** (Savoia et al., 2022)

The reaction mixture used, in a final volume of 25 µl , was composed of: 50 ng / µl of DNA, 1X buffer, dNTP 0.04 M, forward and reverse primer mix 0.25 uM (with forward labeled with fluorophore), DNA Polymerase 0.03 U / µl .

The amplifications were carried out on a C1000 Biorad thermal cycler with a touchdown protocol under the following conditions: 5 minutes at 94 ° C; 10 cycles from: 30 sec at 94 ° C, 45 sec at 55 ° C (annealing temperature), 1 min at 72 ° C; 25 cycles from: 30 sec at 94 ° C, 45 sec at 50 ° C (annealing temperature), 1 min at 72 ° C; final phase: 30 min at 72 ° C; stand-by at 10 ° C.

5. ***Pero*** (Sehic et al., 2012)

The reaction mixture used, in a final volume of 12.5 µl , was composed of: 10 ng / µl of DNA, buffer 1X, dNTP 0.2 mM , forward and reverse primer mix 0.25 uM (with forward labeled with fluorophore), DNA Polymerase 0.025 U / µl .

The amplifications were carried out on a C1000 Biorad thermal cycler with a touchdown protocol under the following conditions: 5 minutes at 94 ° C; 10 cycles from: 30 sec at 94 ° C, 45 sec at 55 ° C (annealing temperature), 1 min at 72 ° C; 25 cycles from: 30 sec at 94 ° C, 45 sec at 50 ° C (annealing temperature), 1 min at 72 ° C; final phase: 15 min at 72 ° C; stand-by at 10 ° C.

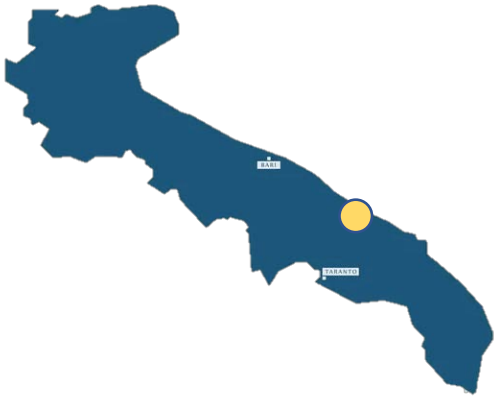
Capillary electrophoresis and obtaining allelic profiles

Following PCR amplification, the samples were prepared to undergo capillary electrophoresis using the 3100 Avant automatic sequencer . Genetic Analyzer (Life technologies). The sequencing preparation contained: 2 µl of each amp; 10.5 µl of formamide ; 0.5 µl Liz 600TM. Finally, a denaturation cycle was performed at 95 ° C for 5 minutes.

The visualization of the fragments is achieved thanks to the use of primers marked with out-of- holes (Osborn et al .; 2000) which are fluorescent molecules that allow the detection of the DNA fragments to which they are bound, by laser reading during a capillary electrophoretic stroke. GeneScan - 600LIZTM was added as standard to the mix used for the sequencer run , covering a " range " of 20 to 600 base pairs (bp). The presence of the standard allows to identify the amplification peaks with greater precision, as the comparison of the peaks obtained with the LIZ and those produced by the samples, allows to obtain a precise value of the length of the amplified sequences and therefore of the alleles that characterize the cultivars. . The graphical acquisition of the electropherograms was carried out using the GENMAPPER software version 3.7 (Applied Biosystems).

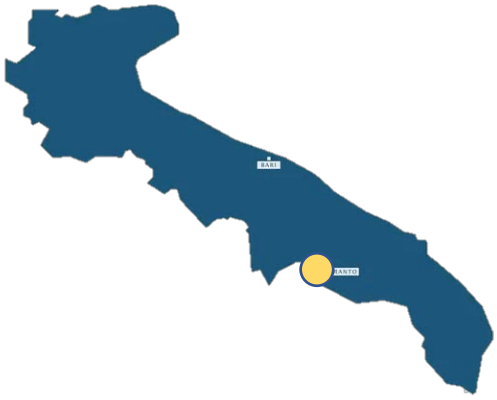
2.0 RESULTS

2.1 Varietal sheets

RGV	**HERBACEOUS**
Genetic resource	**Purple Bean**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

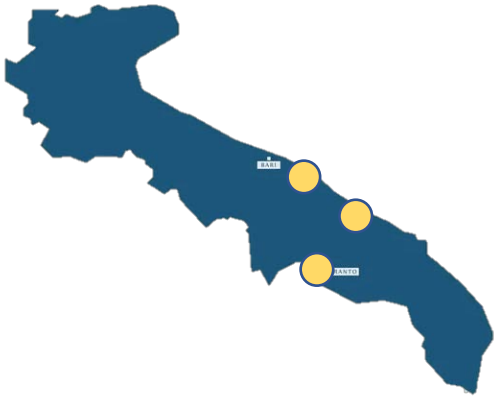
Plant	Determined growth, height of about 50-70 cm, anthocyanin pigmentation of the stem.
Leaves	Medium dark green color.
Flowers	On average 2 flowers per plant, wings with black patches of melanin; anthocyanin banner with patches of melanin, flowering about 138 days after sowing
Fruits	Pod: semi-erect horizontal posture, average length 11-14 cm, slight or absent curvature; 2-3 pods per node; Seed: elliptical shape, violet color, presence of black pigmentation of the hilum.
Production characteristics	Low productivity, but appreciated for its organoleptic characteristics. Crop cycle from November to July.
Other technological features	Farmers define it as more tender and flavorful than the more popular commercial varieties. Some characters related to the quality of the dry grain have been detected. [Weight 100 seeds (g) 210 - 250 Integument (g / 100 g) 14.6 - 14.7 Hydration index (% at 24 h) 118 - 121 Swelling index (% at 24 h) 130 - 140 Proteins (g / 100 gss) 25.3 - 25.4 Ash (g / 100 gss) 5.2

	Total polyphenols (mg GAE / gss) 7.21 - 7.88 Total flavonoids (mg CAE / gss) 1.6 Condensed tannins (mg CAE / gss) 0,8)]

RGV	HERBACEOUS
Genetic resource	**San Pasquale durum wheat**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources CNR-IBBR, Via Amendola 165 / A 70126 Bari; Experimental Didactic Center “P. Martucci "of the Department of Plant and Food Soil Sciences (DiSSPA), University of Aldo Moro di Bari, Via Amendola 165 / A - 70126 Bari,
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

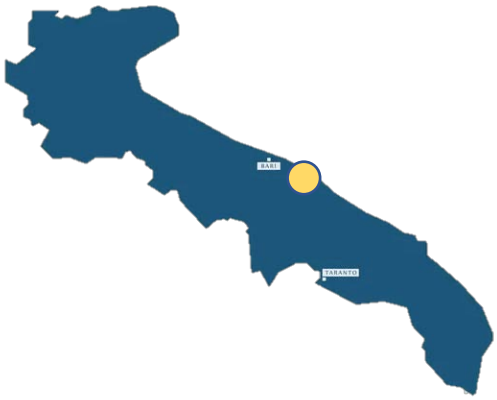
Plant	erect bearing; early emergence of the ear; medium frequency of plants with flag-shaped daughter; absent or very weak coleoptile anthocyanin colouration; height 95-110 cm, whitish awns longer than the ear. Culm: absent or very weak pubescence of the upper node; glaucescence of the culm between the flag leaf and the base of the spike absent or very weak;
Leaves	Flag leaf: glaucescence of the sheath and of the flap absent or very weak
Flowers	Spiga: fusiform shape; absent or very weak glaucescence; absent or very weak anthocyanin pigmentation of the anthers; short length; whitish color when ripe;
Fruits	Gluma: lower gluma from ovoid to elongated with erect and medium-wide shoulder; short straight mucrone; absence of pubescence of the outer surface; Seed: semi-elongated with medium length hairs at the tip; no or very slight phenol colouration
Production characteristics	Type of development: alternative - Earing period (days from 01.04): 12-28 - Ear production: 2.73-3.36 g - Weight of a thousand seeds: 44-46 g

	RESISTANCES - Cold (scale 0-9): 4-5 - Enticement to harvest (scale 0-9): 3-7 - Sick white (scale 0-4): 4
Other technological features	

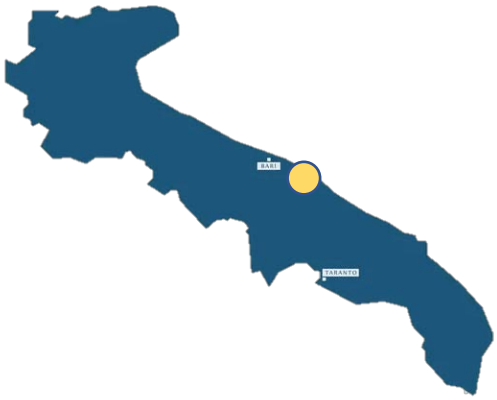
RGV	HERBACEOUS
Genetic resource	**Soft wheat Bianchetta**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources CNR-IBBR - Via Amendola 165 / A 70126 Bari; Experimental Company "Manfredini" CREA Research Center for Cereal and Industrial Crops of Foggia Registered Office Via Po, 14 - 00198 Rome (Italy), Operational Headquarters SS 673 km 25 + 200 - 71122 Foggia
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	Semi-erect posture; period of emergence of the ear from medium to late; medium-high frequency of flag leaf plants; absent or very weak coleoptile anthocyanin colouration; height 110-150 cm, whitish awns longer than the ear, awns or beards both absent or beards present; beards from very short to court. Culmus with absent or very weak upper node pubescence; glaucescence of the culm between the flag leaf and the base of the ear from absent or very weak to medium
Leaves	Absent or very weak to weak glaucescence of sheath and flap;
Flowers	Spiga: fusiform shape with parallel edges; white; absent or very weak to weak glaucescence; absent or very weak anthocyanin pigmentation of the anthers; medium to long length; slightly colored when ripe
Fruits	Gluma: lower gluma with narrow to medium shoulder width and sloping to slightly sloping shape; short to medium mucrone with straight to semi arched shape; pubescence of the external surface from absent to little extended Seed: white; no or very slight phenol coloring

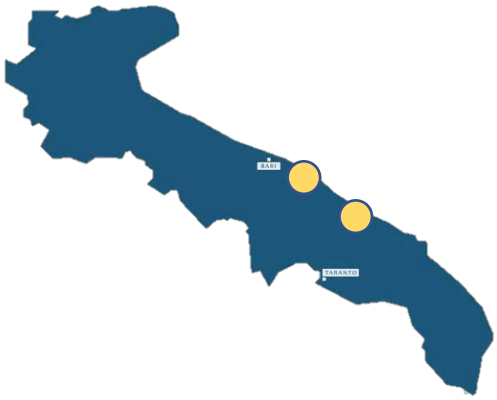
Production characteristics	Type of development: winter components of production and resistance to phosiopathies detected for the 2015/16 and 2016/17 vintages. - Earing period (days from 01.04): 32-46 - Ear production: 1.4-2.2 - Thousand seeds weight: 31-57.9 g RESISTANCES - Cold (scale 0-9): 4-5 - Enticement to harvest (scale 0-9): 6-7 - Sick white (scale from 0-4): 4
Other technological features	INTEGRAL FLOURY CHARACTERS Proteins (g / 100 gss) 13.2-16.3 Yellow index (b *) 8.8-10.3 Brown index (100-L) 12-14.4 Gluten index (%) 1 38-48 Carotenoids (µg / g) 3.3-3.8 Polyphenols (mg ferulic acid / gss) 1,2

RGV	FORAGE
Genetic resource	**Underground clover**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	Plant - Height: 15 - 25 cm - Posture: semi-prostrate
Leaves	Central leaf: medium to large triangular in shape, wider than long Color: intermediate to dark green Indentation of the distal margin: none to much small Pubescence: dense especially on the upper side Leaf markers: small whitish spot in the center more or less evident whitish leaves or arms which embrace ¾ or the entire width of the leaf Anthocyanin redness: weak or absent Pubescence of the petiole: dense Anthocyanin coloration of the stipules: only on the veins or in a wide band covering from half to the entire stipule
Flowers	- Color: white with faint pinkish veins - Stem pubescence: very dense - Flowering period: from the end of March to the end of April
Fruits	Seeds - Color: purple-black - Shape: globose or ellipsoidal - Glomeruli: spread throughout the plant - Weight of 1000 seeds: 7.4 - 10.4 g
Production characteristics	Duration of the crop cycle from September to May.
Other technological features	MAIN COMPONENTS PHENOLOGICAL STAGE: Vegetative and Flowering. Crude protein (g / 100gss) 27.6 ± 1.3 24.9 ± 0.9 Crude fiber (g / 100gss) 24.0 ± 1.2 25.4 ± 2.4 Crude lipids (g / 100gss) 2.0 ± 0.3 1.7 ± 0.2 Ash (g / 100gss) 14.2 ± 1.4 13.1 ± 1.2 Nitrogenated extractives (g / 100gss) 32.3 ± 3.3 35.0 ± 2.6

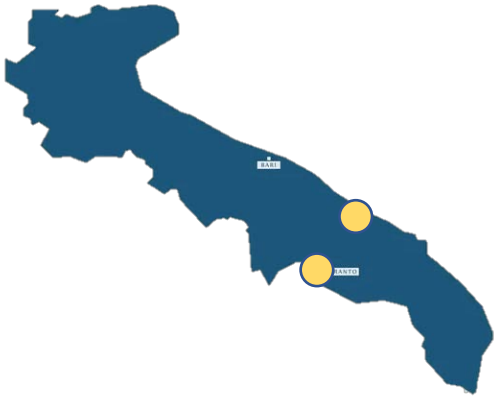
RGV	FORAGE
Genetic resource	**Incarnate clover**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	- Average height: 70 cm - Posture: erect - Hairiness of the stem: medium
Leaves	- Color: intermediate green - Shape: rounded with indentation at the apex - Perforation: absent
Flowers	Flower - Color: purple - Flowering period: first - second decade of May
Fruits	Seeds - Color: yellow-brown - Shape: oval - 1000 seeds weight: 3.2 - 3.6g
Production characteristics	Duration of the crop cycle from October to June.
Other technological features	MAIN COMPONENTS Crude protein (g / 100gss) 17.1 Crude fiber (g / 100gss) 25.3 Ash (g / 100gss) 9.3 Nitrogenated extractives (g / 100gss) 47.3

RGV	FRUIT
Genetic resource	**Petrelli fig**
Conservation bodies	Department of Soil, Plant and Food Sciences of the University of Bari Aldo Moro, Via Amendola 165 / A, 70126 Bari; Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA); Foundation for the management of the University Botanical Garden of Lecce - 73100 Lecce, Masseria S. Angelo
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

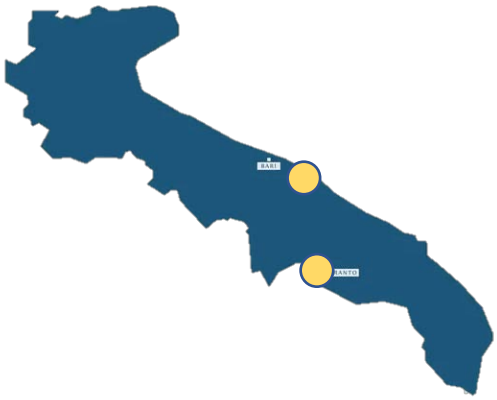
Plant	Plant: high vigor; expanded habit with dense branching; medium aptitude to produce suckers.
Leaves	dimensions 28.8 cm x 20.1 cm; five-lobed shape with crenate margin; dark green color; central oboval lobe; circular ovate lateral lobes; open petiolar sinus; long petiole with dimensions> 80 mm of light green color;
Flowers	(see fruits)
Fruits	parthenocarpic development; high weight (> 90 g); very large width (> 60 mm); high length (> 75 mm); piriform shape; hemispherical apex; presence of a small neck; easy detachment of the fruit from the peduncle; high leakage of latex from the peduncle; depressed and semi-open ostiole, of average size 1-3 mm; white color of the ostiol; absence of pink drop at the opening of the ostiole; peel with a green background color; absence of overcolour of the peel; peel thickness 2-3 mm; ease of peeling; transverse cracks in the peel; abundant bloom; medium present lenticels of medium size; dark red pulp of medium

	texture; aromatic flavor; medium presence and size of achenes; high juiciness and sweetness;
Production characteristics	Harvesting period: first and second ten days of August; Highly productive, medium ripening scale. It has no particular agronomic needs. Resistant to drought and salty soils.
Other technological features	Poor resistance to manipulation, especially of those supplied. Variety with a sour and aromatic flavor, very pleasant. Only suitable for fresh consumption. Get into many traditional recipes. in the Fasano area, the fiorone of this variety is also tasted rubbed on hot bread together with walnuts or accompanied by capocollo and almonds.

RGV	FRUIT
Genetic resource	**Verdesca fig**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA); Foundation for the management of the University Botanical Garden of Lecce - 73100 Lecce, Masseria S. Angelo
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

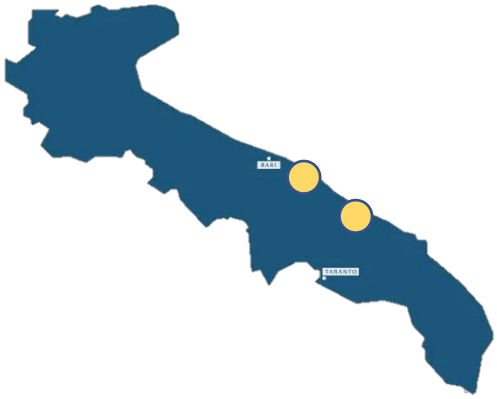
Plant	Plant: high vigor; expanded habit with dense branching; medium aptitude to produce suckers.
Leaves	Leaf: size 21cm x 19cm; five-lobed shape with crenate margin; dark green color; central oboval lobe; circular ovate lateral lobes; open petiolar sinus; petiole of medium length 50-80 mm of light green color; late fall of the leaves.
Flowers	(see fruits)
Fruits	Fruit: parthenocarpic development; average weight (50-90 g); average width 39-49 mm); average length (47-54 mm); globular shape; flat apex; absence of the neck; easy detachment of the fruit from the peduncle; high leakage of latex from the peduncle; depressed and semi-open ostiole, of average size 1-3 mm; ostiol pink color; presence of pink drop at the opening of the ostiole; peel with a green background color; absence of overcolour of the peel; medium skin

	thickness 2-3 mm; ease of peeling; transverse cracks in the peel; abundant presence of bloom; medium-sized and large white lenticels; dark red pulp of fine texture; intense flavor and high juiciness and sweetness; high presence of medium-sized achenes;
Production characteristics	Harvest period: first ten days of September. Highly productive, medium ripening scale. It has no particular agronomic needs.
Other technological features	High resistance to manipulation. Variety with a sour and aromatic flavor, very pleasant.

RGV	FRUIT
Genetic resource	**Real Gentile Pear**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA)
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

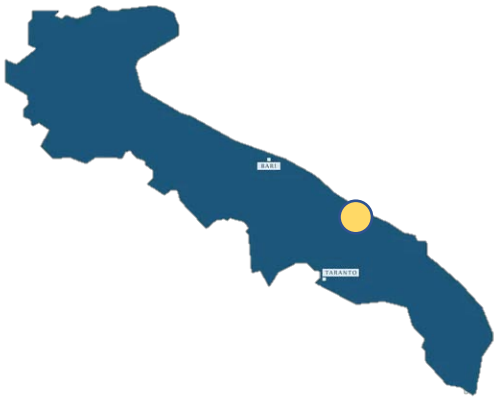
Plant	medium vigor; expanded habit with medium branching; upright-planar branches; fruiting mainly on the lamburde;
Leaves	downward with respect to the shoot; medium size (30-40 cm2); elliptical shape; acute base and acute apex; long apex; crenate margin; superficial incisions on the margin of the leaf blade; dark green top page; absence of pubescence of the lower side; medium length of the petiole; short distance of the stipules from the base of the petiole;
Flowers	medium size of the flower buds; petals separated from each other; medium size of the petals with rounded shape; stigma located inferior to the stamens
Fruits	short turbinate form; Bruno; asymmetrical; maximum diameter towards the glass; small size (110-150 g); concave sides; shallow pedicle cavity (<0.20); not very wide pedicle cavity; disjoint sepals; calycin cavity absent; smooth, uncut, green-yellow skin; low extension of pink-red blush; scarce presence / absence of rust at the attack of the peduncle; absence of rust on the top and bottom; medium length and thickness of the peduncle; medium thick peel; whitish pulp,

	medium texture, firm consistency; medium juiciness and high oxidation; intermediate flavor and medium acidity; seeds of small size (6-7 mm), oval and light brown in color;
Production characteristics	Harvesting period: third decade of June - first decade of July. Highly productive. It adapts to all regional pedoclimatic environments, rustic variety, moderately resistant to scab.
Other technological features	Good size, but poor resistance to handling. Very good flavor, sweet, with a slightly acidic aftertaste. Suitable for fresh consumption, but also for processing into juice, puree, jam etc.

RGV	FRUIT
Genetic resource	**But false Recchia**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA); Foundation for the management of the University Botanical Garden of Lecce - 73100 Lecce, Masseria S. Angelo
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

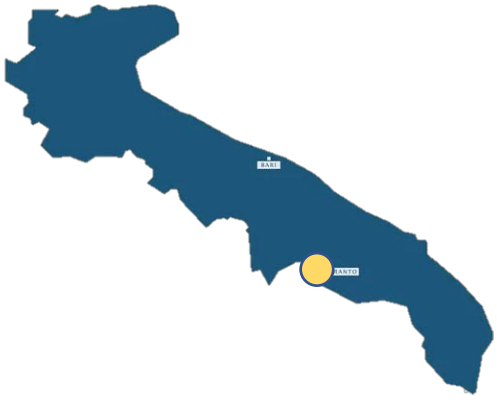
Plant	medium vigor; expanded habit with medium branching; upright-planar branches; fruiting mainly on the lamburde;
Leaves	flat with respect to the shoot; medium size (30-40 cm2); obovate shape; obtuse base and acute apex; long apex; crenate margin; superficial incisions on the margin of the leaf blade; dark green top page; absence of pubescence of the lower side; long petiole; short distance of the stipules from the base of the petiole;
Flowers	medium size of the flower buds; petals separated from each other; medium size of the petals with an elliptical-elongated shape; stigma located inferior to the stamens;
Fruits	short turbinate form; green-brown; slightly asymmetrical; maximum diameter towards the glass; small size (110-150 g); concave sides; shallow pedicle cavity (<0.20); not very wide pedicle cavity ; overlapping sepals; calycin cavity absent; smooth, uncut, green-yellow skin; no or very limited overcolour; large presence of rust at the attack of the peduncle; medium presence of rust on the upper and lower part; medium length and thickness of the peduncle; thin peel; whitish

	pulp, coarse texture, medium consistency; dry and high oxidation; intermediate flavor and low acidity; seeds of small size (6-7 mm), oval and light brown in color
Production characteristics	Harvesting period: third decade of July - first decade of August. Highly productive. It adapts to all regional pedoclimatic environments, rustic variety, moderately resistant to scab.
Other technological features	Medium resistance to manipulation. Very good flavor, sweet, with a slightly acidic aftertaste. Suitable for fresh consumption, but also for processing into juice, puree, jam etc. having a fairly high sugar content, so it requires very little added sugar.

RGV	FRUIT
Genetic resource	**Plum S. Anna Ovale**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA)
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

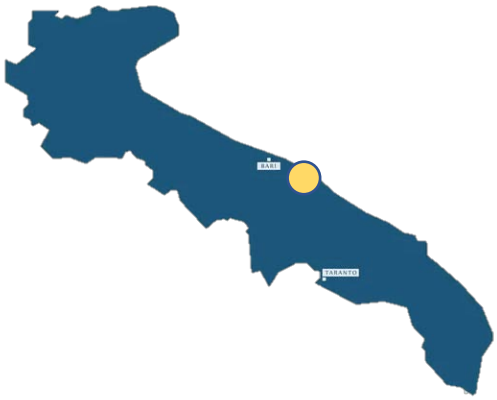
Plant	Erect branches and darts;
Leaves	small leaves; length 60 mm; width 32 mm; obovate shape; obtuse apex and acute base; medium pubescence of the lower side; tight edge; dark green top page; light green bottom page; medium length of the leaf petiole; absence of foliar glands
Flowers	small corolla; sepals of ovate shape; elliptical and elongated petals in contact with each other; absence of pubescence of the ovary
Fruits	Medium size; average weight 80 g; length 70 mm; width 41 mm; thickness 35 mm; elliptical shape; rounded apex; shallow pedicle cavity and not very wide; very noticeable light-colored suture line; medium detachment of the peduncle from the fruit; apex of the peduncle after dry detachment; golden yellow epicarp; absence of overcolour; thin peel; high number of lenticels on the skin; medium-sized lenticels; average firm pulp; aromatic flavor; semi-sticky stone to the pulp; average amount of juice produced; colorless juice; medium-high acidity.

Production characteristics	Harvest period: end of July Highly productive, medium ripening scale. It has no particular agronomic needs.
Other technological features	Medium resistance to manipulation. Variety with a sour and aromatic flavor, very pleasant.

RGV	ORTIVE
Genetic resource	**Taranto White Artichoke**
Conservation bodies	Experimental Didactic Center "P. Martucci "of the Department of Soil, Plant and Food Sciences (DiSSPA), Aldo Moro University of Bari, Via Amendola 165 / A - 70126 Bari; National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

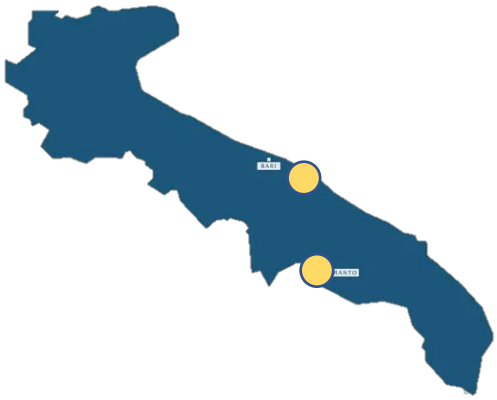
Plant	main stem of small dimensions with two lateral heads; height with main flower head of 95 cm; diameter of 120 cm; average polloniferous aptitude.
Leaves	semi-erect attitude; length of 75 cm; greyish green color; faint reddish color at the base of the midrib.
Flowers	Main flower head: height of 11 cm; diameter of 7 cm; weight 120-190 g; oval longitudinal section; flat apex; average density of the internal bracts. External bracts: green color of the external side; acute apex; thorn absent or very short; longer than wide shape.
Fruits	na
Production characteristics	Harvest period: March-May. The plant produces 5-6 flower heads and can be productive for more than three years. average polloniferous attitude

Other technological features	Chlorogenic acid and cynarin are the most present antioxidants, albeit in lower quantities compared to other local Apulian varieties analyzed.

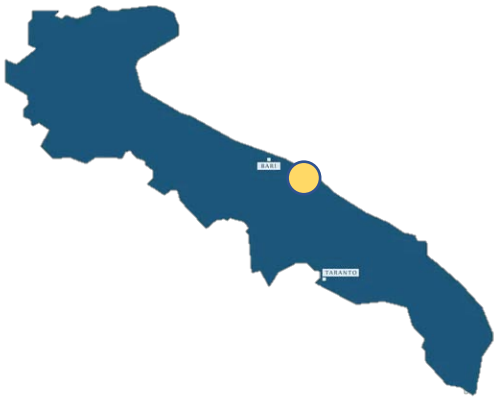
RGV	ORTIVE
Genetic resource	**Top of Cola**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	uniform morphotype; during growth it has an elongated unbranched stem that ends with an enlarged flolal or prefloral apex. Height 75 cm, diameter 80 cm. Triangular root of medium length 15-20 cm x 3-4 cm.
Leaves	leaf length 70 cm, leaf blade width 35 cm, leaf angle open about 67 °, leaf blade ovate, intermediate leaf tip with medium thick whole leaf blade, medium blistering of the leaf blade, leaf tip turning downwards, blade convex leaves curved upwards, dark green leaf color, broad and light green peduncle and / or central vein, petiole 5 cm x 1.5 cm x 15 mm
Flowers	medium-sized exposed flower head in relation to the size of the plant 20-25 cm x 13-16 cm, leaves forming the flower head curved outwards, external leaves of the flower head dark green, flower head of intermediate consistency, internal cut light green, presence of axillary buds that remain dormant, compact flower head consisting of irregularly arranged supcapolini. spherical longitudinal section of the inflorescence, wide and deep inflorescence with a yellow surface, absence of bracts in the inflorescence, low predisposition to early flowering, medium length of

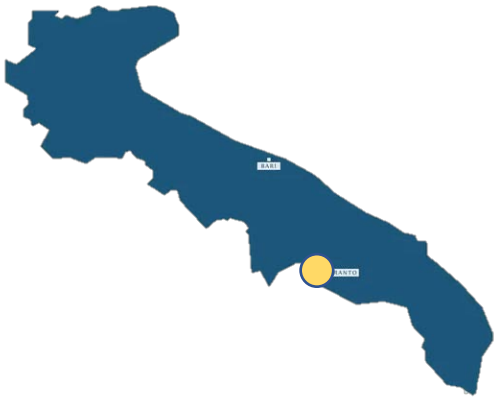
	the flower peduncle, medium branched flower stem, uniform yellow flower
Fruits	3-5 cm x 0.3-0.4 siliqua with upright attitude and narrow edge between seeds, rostrum on average 5 cm long, few seeds per siliqua (10 or less) with brown integument
Production characteristics	Harvest period: from October to January
Other technological features	The edible part of Cima di cola is more spongy than the cauliflower varieties on the market and gives off a strong odor during cooking.

RGV	ORTIVE
Genetic resource	**Occhiopinto cowpea**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	erect-acute growth, moderate pigmentation at the base and tip of the petiole,
Leaves	globular terminal leaflet, presence of glabrescence.
Flowers	flowering about 26 days after sowing, raceme positioned between the canopes, white color
Fruits	Pod: maturation about 91 days after sowing, pod hanging from the peduncle, pigmented tip of the immature pod, slightly curved ripe pod about 14.5 cm long and about 0.74 cm wide, presence of about 11 niches per pod, mature pod of light brown or straw color. Seed: ovoid shape, head from rough to wrinkled, small eye that turns from blue to black, cream colored integument, length of about 7.5 mm, width of about 5.5 mm.
Production characteristics	Harvest period: May-June
Other technological features	Much appreciated in Puglia and is used in recipes linked to local tradition.

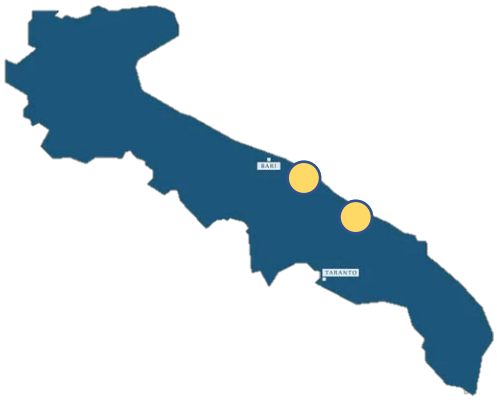
RGV	ORTIVE
Genetic resource	**Pinto green bean**
Conservation bodies	National Research Council, Institute of Biosciences and BioResources (CNR-IBBR), Via G. Amendola 165 / A, 70126 Bari
In vivo conservation	Test fields
In vitro conservation / germplasm banks	Germplasm banks
Possible start-up in BEST areas	

Plant	climbing type growth, very light pigmentation,
Leaves	globular terminal leaflet, presence of glabrescence.
Flowers	flowering about 58 days after sowing, raceme positioned between the canopes, white color.
Fruits	Pod: maturation about 87 days after sowing, pod hanging from the peduncle, pigmented tip of the immature pod, slightly curved mature pod about 31 cm long and about 0.81 cm wide, presence of about 21 niches per pod, mature brown pod dark. Seed: kidney-shaped, smooth head, brown integument, length about 11 mm, width about 6.1 mm.
Production characteristics	Harvest period: May-June. Narrow and very long pods (up to 100 cm), green in color and with an average production. It is rustic.
Other technological features	Much appreciated in Puglia, although now very rare. The culinary preparations used in Puglia are the same as the other non-climbing varieties of pinti green beans.

RGV	LIVES
Genetic resource	**Cigliola vine**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA)
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

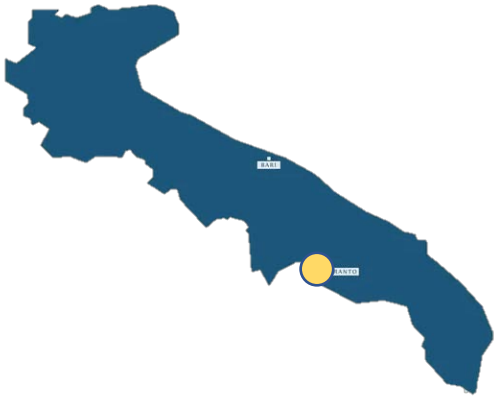
Plant	erect bearing; tendrils distributed on the shoot in a discontinuous manner; dorsal and ventral side of the internodes slightly striped green;
Leaves	young leaf of green-pink color with a strong density of the hairs crawling between the ribs of the lower side; Adult leaf: medium-small size; pentagonal flap; presence of five weakly depressed lobes; anthocyanin pigmentation of the main veins only at the petiolar point; flat profile; medium blistering of the upper side of the flap; convex teeth of medium size; open petiolar sinus; absence of teeth on the edge of the petiole sinus; low density of the creeping hairs between the main ribs (lower page); low density of upright hairs between the main ribs (lower page);
Flowers	Flower bud with open end; absence of anthocyanin pigmentation of the crawling hairs of the extremity; high density of the crawling hairs of the extremity;

	Inflorescence: hermaphrodite flower; inflorescences per shoot from 2 to 3; high fertility of the basal buds of the shoot;
Fruits	Ripe bunch: medium length and compactness; conical shape; Berry when ripe: medium-short size; ellipsoidal shape; green-yellow epidermis; thick peel; slightly firm pulp; presence of seeds;
Production characteristics	Medium vigor of the shoot; medium length of internodes; medium-low weight of the grape and bunch; average grape production per m2; high or very high sugar content of the must; low or very low total acidity of the must; very high must pH; characterized by early phenological phases, starting from bud break; the flowering, veraison and finally ripening phases occur early. Productivity is regular and constant, fertility is good
Other technological features	The wine obtained is characterized by a straw yellow color, quite intense, limpid, has a moderate olfactory intensity characterized above all by floral (rose, violet) and herbaceous notes based on fresh grass, hay and also sweet almond, while light but very pleasant are the fruity scents, especially apricot and peach. The good alcohol content and structure are accompanied by an excellent balance and gustatory persistence, so the vine is very well suited as a base for table wines to be accompanied preferably with fish-based dishes.

RGV	LIVES
Genetic resource	**Lives Notardomenico**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA)
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

Plant	semi-erect posture; tendrils distributed on the shoot in a discontinuous manner;
Leaves	Adult leaf: large size; orbicular pentagonal flap; presence of five weakly depressed lobes; absence of anthocyanin pigmentation of the main veins; flat or slightly wavy profile; very light blistering of the upper side of the flap; convex teeth; absence of teeth on the edge of the petiole sinus; absence of creeping hairs between the main ribs (lower page); low density of upright hairs between the main ribs (lower page);
Flowers	Sprout when flowering: open end; absence of anthocyanin pigmentation of the crawling hairs of the extremity; light-medium density of the crawling hairs of the extremity; dorsal side of the internodes slightly streaked green; young leaf of a slightly pinkish green color with low density of the hairs crawling between the ribs on the underside;

	Inflorescence: hermaphrodite flower; presence of one or two inflorescences per shoot; medium fertility of the basal buds of the shoot;
Fruits	Bunch when ripe: high length; loose bunch; peduncle of medium length; cylindrical shape; Berry when ripe: high size; spheroidal shape; black-purple epidermis; thin peel; uncolored pulp; high consistency of the pulp; presence of seeds;
Production characteristics	very high shoot vigor; medium length of internodes; high weight of the grape; high production of grapes per m2; medium sugar content of the must; average total acidity of the must; low pH of the must; The Notardomenico is characterized by a bud break in the middle period; the other phases of flowering, veraison and maturation take place in an average period. High fertility, both basal and distal, and productivity.
Other technological features	it lends itself very well to obtaining a fine rosé wine, already produced in ancient times in the areas where it was grown. The wine vinified in red has a ruby red color, not very intense, but bright, characterized by a good aromatic complexity with a prevalence of notes of ripe fruit, in particular red fruits. The overall balance is fair, while the structure is weak, so the wine is not suitable for aging.

RGV	LIVES
Genetic resource	**Vine Santa Teresa**
Conservation bodies	Research Center, Experimentation and Training in Agriculture Basile Caramia, Via Cisternino, 281 - 70010 Locorotondo (BA)
In vivo conservation	Collection fields
In vitro conservation / germplasm banks	Cryopreservation of meristematic apices
Possible start-up in BEST areas	

Plant	erect bearing; tendrils distributed on the shoot in a discontinuous manner;
Leaves	Adult leaf: small size; wedge-shaped flap; presence of five weakly depressed lobes; absence of anthocyanin pigmentation of the main veins; revolute profile; absence of blistering of the upper side of the flap; medium long straight teeth; absence of petiolar sinus; absence of teeth on the edge of the petiole sinus; absence of creeping hairs between the main ribs (lower page); absence of erect hairs between the main ribs (lower page);
Flowers	Sprout when flowering: open end; medium anthocyanin pigmentation of the creeping end hairs; low density of the creeping hairs of the extremity; dorsal side of internodes green with red streaks; young leaf of green-pink color with low density of the hairs crawling between the veins of the lower side; Inflorescence: hermaphrodite flower; high number of inflorescences per shoot; high fertility of the basal buds of the shoot;

Fruits	Ripe cluster: long and compact cylindrical cluster; Ripe grape: small size; spheroidal shape; green-yellow epidermis; thick peel; uncolored pulp; slightly firm pulp; presence of seeds;
Production characteristics	high vigor of the shoot; medium length of internodes; high weight of the bunch; medium heavy grape; high production of grapes per m2; low sugar content of the must; average total acidity of the must; medium pH value of the must; late budding; the other phases of flowering, veraison and ripening take place in a late period. Good fertility, both basal and distal, and productivity Harvest: late (first ten days of October)
Other technological features	The wine has a straw yellow color of good intensity. Good aromatic complexity mainly due to aromas of fermentation origin. With a fairly low alcohol content, it reveals a good content of total acidity, which makes the overall balance discreet and with a good intensity and gustatory persistence. On the palate, despite a somewhat poor structure, it is equally appreciated above all for the right balance between the acid flavor and a fair fullness of the body.

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2.2 The allelic profiles obtained.

The molecular analysis was carried out on the arboreal plants, because these plants are more easily and stably identifiable in the areas analyzed. After many years, the tree plant is always present, vice versa the herbaceous and horticultural plants are not always available and are planted according to the needs of the farmers.

In the characterization phases, the molecular investigation is the first that is carried out, because it allows to easily identify cases of homonyms (plants that have the same name but are actually different genotypes) and synonymies (plants called differently but which are the same genotype). This screening work significantly reduces the number of samples to be analyzed at a morphological level and provides guarantees on the correct identity of the material.

Molecular analysis, in addition to identifying a genotype, allows to analyze the level of genetic diversity present in the areas analyzed. For this reason and also to have a greater reliability of the size of the selected sample, supernumerary profiles of other genotypes found in the analyzed areas were included.

The allelic profiles expressed in base pairs (bp) obtained by microsatellite analysis are shown below for each variety examined (Table 3-8).

PCR amplifications using SSR markers and the subsequent capillary electrophoresis step gave clear results in all examined species.

Most of the species analyzed show the presence of two alleles at each microsatellite locus , as these are characterized by a diploid chromosomal arrangement (fig, vine, Indo-Japanese plum , almond and pear). On the contrary, the European plum tree shows more than two alleles for the microsatellite locus examined, being characterized by a polyploid chromosomal set.

Table 3. Allelic profiles obtained from the analysis using SSR markers for the examined Fico varieties

Varietà	MFC1		MFC3		MFC25		MFC26		MFC27		MFC28		MFC30		MFC31		MFC36		MFC37		MFC38		Cup27-4		Cup38-6	
Fico Verdesca	131	181	190	192	230	236	146	162	202	212	114	120	258	270	244	244	241	243	222	224	231	231	202	206	168	176
Fico Petrelli	197	207	180	190	228	234	162	172	202	212	144	210	270	270	244	258	241	241	222	222	235	237	202	204	176	184

Table 4. Allelic profiles obtained from the analysis using SSR markers for the Sant'Anna plum variety.

Varietà	CPDCT025					CPSCT018					UDP98-409						CPPCT006				UDP96-005				BPPCT001			
Susino Sant'Anna	172	176	184	196	212	135	145	155	163	219	121	123	127	137	141	149	181	183	185	191	101	105	113	147	122	138	148	190
	UDP98-412					CPSCT012					BPPCT-014						Pchgms1				ps08e08		BPPCT-010		BPPCT025			
	91	99	115	131	140	154	160	166	174	183	187	227	251	255	160	164	170	172	178	176	180	117	135	139	157	159	169	201

Table 5. Allelic profiles obtained from the analysis using SSR markers for the Goccia d'oro plum variety.

Varietà	CPDCT025		CPSCT018		CPPCT006		BPPCT001		BPPCT025		BPPCT007		UDP98412		CPSCT012		BPPCT014		Pchgms1		ps08e08		BPPCT010	
Susino Goccia d'oro	189	194	155	166	193	207	135	137	151	158	120	142	93	93	154	154	187	194	161	171	0	0	127	129

Table 6. Allelic profiles obtained from the analysis using SSR markers for the vine varieties examined

Varietà	VVS2		VVMD5		VVMD7		VVMD25		VVMD27		VVMD28		VVMD32		VRZAG62		VRZAG79	
Vite Cigliola	133	133	225	225	239	239	241	255	182	184	236	244	257	263	188	194	244	248
Vite Notardomenico	133	143	225	225	239	251	241	255	178	182	244	244	251	253	196	200	246	258
Vite Santa Teresa	143	145	225	233	249	253	241	255	178	184	236	258	221	253	188	202	236	250
Vite Ignota Bianca	141	145	225	231	249	253	238	238	173	183	226	234	254	268	190	202	245	261
Vite Piccola nera	131	141	225	235	249	251	236	236	179	179	246	256	252	260	200	204	237	259
Vite Pizzutella	131	149	235	247	241	251	236	236	183	183	242	246	248	258	188	188	249	249
Vite Lattuario	149	155	0	0	249	255	0	0	177	177	0	0	0	0	188	188	247	257
Vite Giulia Ciola	131	141	227	227	241	249	236	236	185	193	232	232	248	268	192	200	257	257
Vite Chiobbica	133	141	225	231	241	251	236	252	177	179	244	246	250	250	188	194	251	259
Vite Sagrone rosso	131	141	241	241	241	253	236	252	179	181	242	246	246	246	188	200	247	259

Table 7. Allelic profiles obtained from the analysis using SSR markers for the almond varieties examined

Varietà																		
Mandorlo_Di_sabato	UDP98-409		CPPCT033		CPDCT025		CPPCT006		CPDCT045		CPSCT018		BPPCT007		UDP98-412		UDP96-005	
	126	148	151	163	194	194	194	202	152	158	151	151	141	141	120	120	128	132
	CPSCT012		BPPCT001		BPPCT025		pchgms1		UDP96003		BPPCT-010		BPPCT-014		EPPCU5176		CPDCT042	
	156	158	0	0	175	177	190	202	97	101	122	160	178	194	122	124	0	0
Mandorlo_Montranese	UDP98-409		CPPCT033		CPDCT025		CPPCT006		CPDCT045		CPSCT018		BPPCT007		UDP98-412		UDP96-005	
	140	140	151	163	178	186	196	198	138	160	0	0	147	155	120	120	126	158
	CPSCT012		BPPCT001		BPPCT025		pchgms1		UDP96003		BPPCT-010		BPPCT-014		EPPCU5176		CPDCT042	
	156	166	145	145	165	177	196	196	95	101	134	142	178	192	116	124	160	198
Mandorlo_Montrone	UDP98-409		CPPCT033		CPDCT025		CPPCT006		CPDCT045		CPSCT018		BPPCT007		UDP98-412		UDP96-005	
	140	140	163	163	194	196	176	194	138	158	151	155	127	147	104	112	136	154
	CPSCT012		BPPCT001		BPPCT025		pchgms1		UDP96003		BPPCT-010		BPPCT-014		EPPCU5176		CPDCT042	
	150	158	131	147	167	177	196	200	97	101	136	156	194	194	124	130	160	168

Varietà														
Pero Gentile Reale	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	91	101	112	116	120	136	141	155	90	90	150	154	155	155
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	122	142	178	178	174	179	204	265	277	277	155	159	135	137
Pero Recchia Falsa	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	93	111	116	116	120	130	141	141	90	90	138	156	172	172
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	122	124	178	178	184	187	247	265	277	281	139	147	131	131
Pero San Cosimo	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	87	111	92	116	128	130	137	141	90	109	138	138	172	172
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	124	136	178	178	187	187	231	265	277	293	147	163	0	0
Pero a sole	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	97	101	107	116	120	126	141	141	90	130	150	154	194	194
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	130	130	178	178	174	186	241	265	277	285	159	163	137	137
Pero Campanello rosso di Ottobre	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	87	91	97	107	120	120	141	141	90	130	150	154	194	194
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	130	130	178	178	174	186	255	265	279	285	159	163	137	145
Pero San Giovanni	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	87	97	88	124	126	130	143	147	107	118	132	132	155	170
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	128	128	178	178	179	188	243	243	275	279	138	181	135	135
Pero Genio	CH05c06		EMPc117		GD147		EMPc11		CH03d12		CH01d09		GD96	
	87	103	114	124	120	130	141	151	118	123	150	154	163	163
	CH02b10		CH04e03		CH01f07a		CH03g07		CH01d08		GD142		CH_Vf1	
	130	140	178	204	179	179	206	241	277	277	151	181	147	147

Table 8. Allelic profiles obtained from the analysis using SSR markers for the pear varieties examined

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